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Research article

ASSESSING RISKS OF SECONDARY IMMUNODEFICIENCY IN CHILDREN WITH ALUMINUM CONTAMINATION IN BIOLOGICAL MEDIA AND POLYMORPHISM OF THE CELL DEATH GENE *FAS RS1159120* AND THE ANTIGEN-RECOGNIZING GENE OF THE TOLL-LIKE RECEPTOR *TLR4* RS1927911

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Secondary immunodeficiency remains an incompletely understood medical problem, and despite a significant number of international and scientific studies, there is no complete picture of the causes and consequences of this pathology. Metal cations have been proven to participate in the formation of acquired immunodeficiency. In particular, aluminum properties as an immune suppressor have been established and its targets in the body have been identified in case aluminum was present in biological media. However, no evaluations have been accomplished so far as regards the role of specific point genetic changes, that is, polymorphisms in the genes of immune system compartments that determine the risk of negative effects caused by contamination with metal cations, including aluminum. It is quite relevant to search and substantiate immunogenetic markers to create an indicator system for diagnostics and prevention of secondary immunodeficiency states in children associated with aluminum contamination in biological media.

We examined 97 preschool children exposed to elevated levels of airborne aluminum (in an area influenced by a metallurgic production). The study groups were divided depending on either presence or absence of secondary immunodeficiency as immune system pathology (common variable immunodeficiency D83). Several markers of the immune system were evaluated: aluminum-specific IgG, $CD3^+$, $CD4^+$, $CD8^+$, $CD127^-$, $CD95^+$, $CD284^+$, and phagocytic activity; we also evaluated polymorphism of TLR4 A8595G (rs1927911) and FAS C14405T (rs1159120) genes of innate and acquired immunity.

According to the results obtained by examining biological media composition, children with secondary immunodeficiency had 1.8 times higher aluminum levels in urine (0.0095 ± 0.0014 vs. 0.0054 ± 0.0009 mg/dm^3, reference range < 0.0075 mg/dm^3) as opposed to their conditionally healthy peers. We established an authentic inverse dependence between the expression level of the main CD clusters ($CD3^+$: r = -0.38; $CD4^+$: r = -0.39; $CD8^+$: r = -0.26) as well as indicators of phagocytic activity (r = -0.22–0.23) and the level of aluminum contamination in biological media (urine). Expression of T-mature lymphocyte clusters was found to be inhibited by 1.3–3.1 times (including T-helpers, effector T-lymphocytes, NK-killers, regulatory lymphocytes) and we also detected some changes in expression of specific immunoglobulin of IgG class to aluminum. All this results in an unacceptable level of relative risk (RR = 1.23–1.63) of developing secondary immunodeficiency against increased frequency of allele C and genotype CC of FAS gene (rs1159120) by 1.2 and 1.5 times respectively, as well as minor allele G of TLR4 gene (rs1927911) by 1.8 times relative to the comparison group (OR = 4.05; CI: 1.41–11.59; p = 0.006; RR = 1.23; CI: 1.02–1.48) and (OR = 2.01; CI: 1.04–3.91; p = 0.037; RR = 1.64; CI: 1.46–1.94). Toll-dependent and FAS-dependent mechanism of this risk is associated with aluminum contamination. It is recommended to use a combination of immune and genetic markers as indicator ones when evaluating the immune system state, in order to prevent the risk (RR = 1.23–1.63) of secondary immunodeficiency associated with aluminum contamination in biological media.

Keywords: *aluminum, children, relative risk, secondary immunodeficiency, cell differentiation clusters, FAS gene, TLR4 gene.*

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Identification of imbalance in microelements, like aluminum, is an important task that has to be tackled to prevent development of chronic intoxications. Aluminum does not have any biological function in the human body; however, when this element or its compounds persist in biological media for a long time, they may affect the hematopoietic system, bones, nervous system and immunological indicators[1] [1, 2]. This metal has adverse effects not only on cell structures but also on their functions, in particular, ability to proliferate [3]. Aluminum promotes the expression of the apoptosis gene *FAS* [4]. D. Cheng with colleagues established and proved that aluminum was able to damage macrophages thus promoting secondary immunodeficiency development [1, 5]. Experimental data reported by B. Wang with colleagues allowed establishing that aluminum could also affect gut microbiota decreasing its diversity and disrupt its overall structure, which, in its turn, had a negative effect on the immune system [6].

Secondary (acquired) immunodeficiency is a specific dysfunction of the immune system that can occur in different age groups. Children aged 4–6 years are exposed to elevated viral and bacterial loads and any disruption of cell differentiation, proliferation and adaptation, together with decline in their number and activity, results in higher respiratory incidence with possible bacterial infections [7].

Although secondary immunodeficiency is not innate, nevertheless, polymorphism of genes that regulate the immune response has a significant role in making the body resistant to chemical and biological environmental exposures.

Toll-like receptors *TLR* belong to the innate immunity. They are the first to recognize ligands of viruses, bacteria, protozoa and fungi and to launch an immune reaction [8]. There is evidence that polymorphism of *TLR* genes, in particular, *TLR4* that is a signaling molecule responsible for cytokine expression, is associated with allergic, infectious and autoimmune diseases [9, 10].

The *FAS*-mediated apoptosis system ($CD95^+$) contributes to physiological and pathological cellular processes, such as differentiation and survival. Studies on mutations in the *FAS* gene can promote better understanding of the pathogenesis of autoimmune diseases and immunodeficiency formation [11]. *FAS* antigen plays the key role in regulating programmed cell death and is expressed on B- and T-lymphocytes [12, 13].

Search for new immunological and genetic markers associated with the development of aluminum-modified secondary immunodeficiency in children is relevant given new challenges and threats that arise due to changes in the sanitary-hygienic situation caused by exposure to new airborne chemicals able to affect human health.

The aim of this study was to assess risks of secondary immunodeficiency associated with specific polymorphism of the cell death gene *FAS* rs1159120 and the antigen-recognizing gene of the toll-like receptor *TLR4* rs1927911 in children with aluminum contamination in biological media.

Materials and methods. We examined 97 children aged between 3 and 6 years who lived in an area influenced by emissions from a non-ferrous metallurgy plant in Western Siberia. The observation group consisted of 50 children aged 5.0 ± 0.3 years with secondary immunodeficiency (common variable

[1] Zaitseva N.V., Dolgikh O.V., Krivtsov A.V., Otavina E.A., Bubnova O.A., Dianova D.G., Bezruchenko N.V., Perminova I.V. Sposob otsenki vliyaniya alyuminiya na immunnyi status [The method for assessing effects of aluminum of the immunity]: patent No. 2629597 Russian Federation, MPK G01N33/53], no. 2016126799; submitted on July 04, 2016; published on August 30, 2017, Bulletin No. 25; Federal Scientific Center for Medical and Preventive Health Risk Management Technologies is the applicant and patent holder (in Russian).

immunodeficiency, D83); the reference group was made of 47 presumably healthy children aged 4.3 ± 0.3 years. Both groups were comparable in terms of age, socioeconomic status and ethnicity.

All examinations were conducted at the Federal Scientific Center for Medical and Preventive Health Risk Management Technologies (the study was approved by the Ethics Committee, the meeting report No. 8 dated July 17, 2023) in conformity with the ethical standards stipulated in the WMA Declaration of Helsinki.

Aluminum was quantified in urine using a quadrupole mass spectrometer with inductively coupled plasma Agilent 7500cx (Agilent Technologies, USA) with the octopole reaction system (ORS) in accordance with the methodical guidelines MUK 4.1.3589-19 "Measurement of aluminum mass concentration in biological media (blood, urine) by inductively coupled plasma mass spectrometry".

Immunological tests of blood serum indicators were performed using the unified research technique, namely, ELISA tests, on a Biotek ELx808 (USA).

We examined CD-clusters of cell differentiation and intracellular apoptosis markers on a flow cytometer BD FACSCalibur (USA) using relevant monoclonal antibodies and the universal software CellQuestPrO. The examinations included determining $CD127^-$, $CD16^+56^+$, $CD19^+$, $CD3^+$, $CD3^+$ $CD8^+$ counts with flow cytometry.

Phagocytic activity levels were identified using formalized sheep erythrocytes as phagocytosis objects following the V.N. Kaplin technique.

We examined polymorphism of the cell death *FAS* rs1159120 gene and antigen-recognizing toll-like receptor *TLR4* gene by real-time polymerase chain reaction performed on a CFX96 Real Time System.

Research data were statistically analyzed using Statistica 12.0 (StatSoft, Inc., USA) and Microsoft Excel 2013. We determined the simple mean (X) and its standard deviation (SD). Distribution was checked for normalcy using the Shapiro – Wilk test. Mean values were compared using the Student's t-test. The relationships between the analyzed immune markers and levels of aluminum contamination in biological media were assessed using Pearson Correlation. Risk assessment involved calculating relative risks (RR) of negative processes associated with candidate gene polymorphism. Differences were considered authentic at the significance level being above 95 % ($p < 0.05$).

Results and discussion. Chemical tests established that children from the observation group had authentically 1.8 times higher aluminum levels in urine against the reference group (0.0095 ± 0.0014 against 0.0054 ± ± 0.0009 mg/dm^3, the reference level is below 0.0075 mg/dm^3).

Specific immune response was established to be authentically higher in the observation group as per the level of IgG to aluminum, which was 1.8 times higher than in the reference group ($p < 0.05$) [14].

Comparative analysis of cell differentiation clusters established an authentic imbalance of regulatory mediators in the observation group associated with immunodeficiency. Thus, the absolute count of T-mature CD3+-lymphocytes responsible for cell-mediated immunity was 1.4 times lower. We established an authentic inverse correlation between CD3+ levels and levels of aluminum contamination in biological media (urine) ($r = -0.38$; $p < 0.05$). Alpatova N.A. with colleagues reported a decrease in the proportion of $CD3^+$-lymphocytes in animals upon aluminum administration[2] [15]. We found defi-

[2] Zaitseva N.S., Sizyakina L.P., Bagmet A.D., Kharitonova M.V. Sposob diagnostiki vtorichnogo immunodefitsita [The method for diagnosing secondary immunodeficiency]: patent No. 2749781 C1 Russian Federation, MPK G01N 33/53, no. 2020137481, submitted on November 16, 2020, published on June 16, 2021; Rostov State Medical University of the RF Ministry of Health is the applicant (in Russian).

ciency of T-helpers $CD3^+CD4^+$ (1.3 times lower in the observation group), which are responsible for antigen recognition and immune response regulation, and also established an authentic inverse correlation with aluminum contamination in biological media (urine) (r = -0.39). In another study, Y. She with colleagues reported less active Th1 immunocytes that secreted pro-inflammatory cytokines under exposure to aluminum [16]. In our study, deficiency of effector T-cytotoxic lymphocytes $CD3^+$ $CD8^+$ (1.4 times lower) was shown to result in cancelled apoptosis launch, which was associated with negative effects produced by aluminum on cellular immunity (r = -0.26; $p < 0.05$) [16, 17]. The present study verifies inhibited production (by 3.1 times lower) of T-regulatory lymphocytes $CD127^-$ as well as an authentic decline in expression of the membrane receptor of *FAS*-receptor cell death $CD95^+$ (by 1.6–1.8 times lower), which is an apoptosis trigger, which control the intensity of an immune response given by T-effector cells [18]. We also established an authentically inhibited expression of the toll-like receptor 4 ($CD284^+$ rel./abs. 1.6–1.8 times lower), which eliminates adequate pro-inflammatory mediator effects of antiviral and antibacterial immunity against the same indicators in the children from the reference group ($p < 0.05$).

Phagocytic activity assessment established that the relative count of phagocytic cells and their absorbing capacity was 10 % lower than in the observation group against the reference one (Table 1). A rise in the aluminum level in biological media (urine) has an inverse correlation with the level of phagocytic activity; this verifies that the chemical is able to suppress immunity[3] [19, 20].

We examined peculiarities of polymorphism of the cell death *FAS* (rs1159120) gene and antigen-recognizing toll-like receptor *TLR4* (rs1927911) gene in children with immunodeficiency exposed to aluminum. As a result, we found significantly ($p < 0.05$) higher frequency of C allele of the *FAS* (rs1159120) gene and its CC genotype, 1.2 and 1.5 times higher accordingly, as well as significantly ($p < 0.05$) 1.8 times higher frequency

Table 1

Peculiarities of the immune profile of the children from the analyzed groups

Indicator	Reference range	Observation	Reference	*p(t)*
IgG to aluminum, atb. units	0–0.1	0.16 ± 0.05	0.09 ± 0.02	***0.0220***
$CD3^+$-lymphocytes, abs., 10^9/l	0.69–2.54	1.54 ± 0.13	2.08 ± 0.24	***0.0000***
$CD3^+CD4^+$-lymphocytes, abs., 10^9/l	0.41–1.59	0.84 ± 0.08	1.10 ± 0.13	***0.0010***
$CD3^+CD8^+$-lymphocytes, abs., 10^9/l	0.19–1.14	0.60 ± 0.07	0.83 ± 0.09	***0.0000***
$CD127^-$ lymphocytes, abs., $10^9/dm^3$	0.015–0.04	0.07 ± 0.03	0.22 ± 0.05	***0.0000***
$CD95^+$ lymphocytes, abs., $10^9/dm^3$	0.43–0.87	0.290 ± 0.04	0.511 ± 0.05	***0.0008***
$CD95^+$ lymphocytes, rel., %	20–40	15 ± 1.05	25 ± 2.15	***0.0000***
$CD284^+$ lymphocytes, abs., $10^9/dm^3$	0.2–0.4	0.208 ± 0.03	0.380 ± 0.04	***0.0008***
$CD284^+$ lymphocytes, rel., %	10–20	11 ± 0.95	18 ± 0.97	***0.0000***
Phagocyte proportion, %	35–60	48.44 ± 1.98	53.76 ± 3.16	***0.0060***
Phagocytic number, atb. units	0.8–1.2	0.88 ± 0.05	1.02 ± 0.08	***0.0070***
Absolute phagocytosis, $10^9/dm^3$	1–2	1.24 ± 0.13	1.93 ± 0.31	***0.0000***

[3] Immunoterapiya: rukovodstvo dlya vrachei [Immunotherapy: guide for physicians]. In: R.M. Khaitov, R.I. Ataullkhanov eds. Moscow, GEOTAR-Media Publ., 2012, 672 p. (in Russian).

Table 2

The results obtained by genotyping of candidate genes *FAS* (rs1159120) and *TLR4* (rs1927911)

Gene	Allele	Observation % (N)	Reference % (N)	$x^2(p)$	OR (CI)	Genotype	Observation % (N)	Reference % (N)	$x^2(p)$	OR (CI)
FAS C14405T (rs1159120)	C	92.4 (85)	75.0 (30)	7.52 (0.006)	4.05 (1.41–11.59)	CC	84.8 (39)	55.0 (11)	7.70 (0.02)	4.56 (1.38–15.03)
						CT	15.2 (7)	40.0 (8)		0.27 (0.08–0.90)
	T	7.6 (7)	25.0 (10)		0.25 (0.09–0.71)	TT	0 (0)	5.0 (1)		0.14 (0.01–3.58)
TLR4 A8595G (rs1927911)	A	72.9 (70)	84.4 (103)	4.34 (0.037)	0.50 (0.26–0.97)	AA	58.3 (28)	73.8 (45)	3.50 (0.17)	0.50 (0.22–1.12)
						AG	29.2 (14)	21.3 (13)		1.52 (0.63–3.64)
	G	27.1 (26)	15.6 (19)		2.01 (1.04–3.91)	GG	12.5 (6)	4.9 (3)		2.76 (0.65–11.68)

of G allele of the *TLR4* (rs1927911) gene against the reference group. The wild C allele of the *FAS* (rs1159120) gene and the minor G allele of the *TLR4* (rs1927911) gene are both factors that determine greater likelihood of unfavorable scenarios (likely manifestations of an immunodeficiency) associated with aluminum contamination in biological media (urine) (OR = 4.05; CI: 1.41–11.59; p = 0.006; RR = 1.23; CI: 1.02–1.48) and (OR = 2.01; CI: 1.04–3.91; p = 0.037; RR = 1.64; CI: 1.46–1.94) (Table 2). The absolute expression of CD3+CD95+ activated T-lymphocytes and CD284+ as markers of apoptosis, cytokine expression and effectiveness of anti-infection immunity was authentically 1.6-1.8 times lower in the observation group in carriers of these polymorphisms of the *FAS* gene (rs1159120) and the toll-like receptor gene *TLR4*. This reflected expected inhibition of cell death and protein elimination controlling as regards both infectious agents and own nonfunctional proteins (Table 2).

Therefore, the present study verifies the hypothesis, which confirms the results reported in previous studies as regards effects produced by aluminum in vitro as immunosuppressant. As an immunosuppressant, the chemical affects certain priority regulatory clusters in cell differentiation, namely, some phenotypes of T-mature lymphocytes, including T-helpers, effector cells, T-regulatory lymphocytes. Among the latter, we should mention those having receptors of apoptosis and cytokine regulation controlling ($CD284^+$; $CD95^+$), imbalance of which is authentically combined with changed frequency of risk-associated alleles of the genes responsible for innate and adaptive immunity, *TLR4* A8595G (rs1927911) and *FAS* C14405T (rs1159120) in children with diagnosed secondary immunodeficiency and aluminum contamination in biological media (RR = 1.23–1.64).

Conclusions. Our study findings indicate that polymorphism of the apoptosis *FAS* rs1159120 gene and antigen-recognizing toll-like receptor *TLR4* rs1927911 gene creates risk (RR = 1.23–1.63) of secondary immunodeficiency in children with elevated aluminum levels in biological media (1.3 times higher than the reference level).

The study reports immune-suppressing effects produced by aluminum on the immune system cells (T-regulatory lymphocytes

$CD127^-$, T-lymphocytes with the $CD95^+$ phenotype, their production is 1.6–3.1 times lower; the $CD284^+$ phenotype of toll-like receptors, production 1.6–1.8 times lower). The effects are verified by authentic models of the relationship between inhibited expression of T-lymphocytic clusters and aluminum levels in urine ($CD3^+$: r = -0.38; $CD4^+$: r = -0.39; $CD8^+$: r = -0.26), which manifests itself as cancelled apoptosis controlling, inhibited activation of its receptor-mediated pathway and anti-infection immunity ($CD284^+$; $CD95^+$).

Authentically higher frequency of candidate alleles and genotypes of innate and adaptive immunity was established; C allele and CC genotype of the *FAS* gene (rs1159120) were 1.2 and 1.5 times more frequent accordingly and minor G allele of the *TLR4* gene (rs1927911) was 1.8 times more frequent than in the observation group. This higher frequency was associated with aluminum contamination in biological media (urine) (OR = 4.05; CI: 1.41–11.59; p = 0.006; RR = 1.23; CI: 1.02–1.48) and (OR = 2.01; CI: 1.04–3.91; p = 0.037; RR = 1.64; CI: 1.46–1.94). This reduces the body resistance to viral and bacterial pathogens in the environment ($CD284^+$) and creates the elevated risk of secondary immunodeficiency (D83).

It is recommended to use immunologic ($CD284^+$; $CD95^+$) and genetic (C allele and CC genotype of the *FAS* (rs1159120) gene and G allele of the *TLR4* (rs1927911) gene) indicators as markers of effect and sensitivity associated with the risk (RR = 1.23–1.64) of aluminum-modified secondary immunodeficiency in children.

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References

1. Skupnevskiy S.V., Ivanov D.V. The effect of aluminum and its compounds on the functions of human organs and tissues (review article). *Vestnik novykh meditsinskikh tekhnologii. Elektronnoe izdanie*, 2023, no. 1, pp. 110–124. DOI: 10.24412/2075-4094-2023-1-3-7 (in Russian).

2. Chelakova Yu.A., Dolgikh O.V., Gusel'nikov M.A., Putilova A.S.A., Galiullina A.S. Indikatornye pokazateli immunnogo i geneticheskogo statusa naseleniya, assotsiirovannye s ekspozitsiei allyuminiem [Indicators of immune and genetic status of the population associated with exposure to aluminum]. *Analiz riska zdorov'yu – 2020 sovmestno s mezhdunarodnoi vstrechei po okruzhayushchei srede i zdorov'yu Rise-2020 i kruglym stolom po bezopasnosti pitaniya: materialy Kh Vserossiiskoi nauchno-prakticheskoi konferentsii s mezhdunarodnym uchastiem: in 2 volumes*. In: A.Yu. Popova, N.V. Zaitseva eds. Perm, Perm National Research Polytechnic University Publ., 2020, vol. 1, pp. 621–625 (in Russian).

3. Yu L., Wu J., Zhai Q., Tian F., Zhao J., Zhang H., Chen W. Metabolomic analysis reveals the mechanism of aluminum cytotoxicity in HT-29 cells. *Peer J.*, 2019, vol. 7, pp. 7524. DOI: 10.7717/peerj.7524

4. Jin C., Liu Q., Wang J., Cai Y. [Effect of aluminium on neural behavior and the expression of Bcl-2 and Fas in hippocampus of weaning rats]. *Wei sheng yan jiu [Journal of hygiene research]*, 2009, vol. 38, no. 1, pp. 1–3 (in Chinese).

5. Cheng D., Zhang X., Tang J., Kong Y., Wang X., Wang S. Chlorogenic acid protects against aluminum toxicity via MAPK/Akt signaling pathway in murine RAW264.7 macrophages. *J. Inorg. Biochem.*, 2019, vol. 190, pp. 113–120. DOI: 10.1016/j.jinorgbio.2018.11.001

6. Wang B., Wu C., Cui L., Wang H., Liu Y., Cui W. Dietary aluminium intake disrupts the overall structure of gut microbiota in Wistar rats. *Food Sci. Nutr.*, 2022, vol. 10, no. 11, pp. 3574–3584. DOI: 10.1002/fsn3.2955

7. Luss L.V. Secondary immunodeficiency in children. The clinician's view on immunomodulators prescription. *Allergologiya i immunologiya v pediatrii*, 2018, no. 4 (55), pp. 4–18. DOI: 10.24411/2500-1175-2018-00017 (in Russian).

8. Lebedeva O.P., Qirko R. Expression of toll-like receptors in the female reproductive tract and its hormone regulation (review). *Nauchnye rezul'taty biomeditsinskikh issledovanii*, 2018, vol. 4, no. 3, pp. 3–17. DOI: 10.18413/2313-8955-2018-4-3-0-1 (in Russian).

9. Diamond G., Legarda D., Ryan L.K. The innate immune response of the respiratory epithelium. *Immunol. Rev.*, 2000, vol. 173, pp. 27–38. DOI: 10.1034/j.1600-065x.2000.917304.x

10. Kovalchuk L.V., Svitich O.A., Gankovskaya L.V., Miroshnichenkova A.M., Gankovskii V.A. The role of Toll-like receptors in pathogenesis of human infection. *Kurskii nauchno-prakticheskii vestnik «Chelovek i ego zdorov'e»*, 2012, no. 2, pp. 147–153 (in Russian).

11. Yamada A., Arakaki R., Saito M., Kudo Y., Ishimaru N. Dual Role of Fas/FasL-Mediated Signal in Peripheral Immune Tolerance. *Front. Immunol.*, 2017, vol. 8, pp. 403. DOI: 10.3389/fimmu.2017.00403

12. Teachey D.T., Lambert M.P. Diagnosis and management of autoimmune cytopenias in childhood. *Pediatr. Clin. North Am.*, 2013, vol. 60, no. 6, pp. 1489–1511. DOI: 10.1016/j.pcl.2013.08.009

13. Sakharnov N.A., Knyazev D.I., Tsvetkova V.D., Solntsev L.A., Utkin O.V. Osobennosti ekspressii mRNK genov FAS-oposredovannogo apoptoticheskogo signalinga pri ostrom infektsionnom mononukleoze u detei do i posle lecheniya [Features of mRNA expression of FAS-mediated apoptotic signaling genes in acute infectious mononucleosis in children before and after treatment]. *Molekulyarnaya diagnostika 2017: sbornik trudov IX Vserossiiskoi nauchno-prakticheskoi konferentsii s mezhdunarodnym uchastiem*, 2017, vol. 2, pp. 489–491 (in Russian).

14. Dolgikh O.V., Otavina E.A., Krivtsov A.V., Zhdanova I.G., Guselnikov M.A., Alikina I.N., Nikonoshina N.A. Immunological markers of children health problems living in the conditions of atmospheric air pollution by aluminum. *ZNiSO*, 2019, no. 2 (311), pp. 15–18 (in Russian).

15. Alpatova N.A., Avdeeva Zh.I., Lysikova S.L., Golovinskaya O.V., Gayderova L.A. General characteristics of adjuvants and their mechanism of action (Part 1). *BIOpreparaty. Profilaktika, diagnostika, lechenie*, 2020, vol. 20, no. 4, pp. 245–256. DOI: 10.30895/2221-996X-2020-20-4-245-25 (in Russian).

16. She Y., Wang N., Chen C., Zhu Y., Xia S., Hu C., Li Y. Effects of Aluminum on Function of cultured Splenic T and D Lymphocytes in Rats. *Biol. Trace Elem. Res.*, 2012, vol. 147, no. 1–3, pp. 246–250. DOI: 10.1007/s12011-011-9307-3

17. Belevtsev M., Sakovich I., Kupchinskaia A., Shytikova M., Parhamovich V., Korosteleva L., Sharapova S., Lipay N. Laboratory diagnostics of primary immunodeficiencies: significance of evaluation of minor lymphocyte subpopulations. *Laboratornaya diagnostika. Vostochnaya Evropa*, 2019, vol. 8, no. 3, pp. 381–393 (in Russian).

18. Dolgopolov I.S., Grivtsova L.Yu., Ustinova O.K., Rykov M.Yu. Primary immunodeficiency in a patient with Kabuki syndrome. *Rossiiskii vestnik perinatologii i pediatrii*, 2022, vol. 67, no. 6, pp. 104–112. DOI: 10.21508/1027-4065-2022-67-6-104-112 (in Russian).

19. Mishina M.N. Pervichnye immunodefitsity [Primary immunodeficiencies]. *Krasota i Meditsina*, 2021. Available at: www.krasotaimedicina.ru (August 19, 2024) (in Russian).

20. Otavina E.A., Dolgikh O.V., Luzhetsky K.P. Osobennosti immunnogo statusa zhenshchin s izbytochnoi massoi tela v usloviyakh kontaminatsii biosred alyuminiem [Features of the immune status of women with excess body weight under aluminum contamination of biological media]. *Analiz riska*

zdorov'yu – 2024: materialy XIV Vserossiiskoi nauchno-prakticheskoi konferentsii s mezhdunarodnym uchastiem: in 2 volumes. In: Prof. A.Yu. Popova, Acad. RAS N.V. Zaitseva eds. Perm, Perm National Research Polytechnic University Publ., 2024, pp. 206–210 (in Russian).

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